Species Diversity, 1997, 2, 167–178

Taxonomic Study on the Eutardigrada from the Northern Slope of Mt. Fuji, Central Japan.
III. Families Macrobiotidae and Milnesiidae[1]

Masamichi Ito

Kiso Experiment Station, Forestry and Forest Products Research Institute,
5473 Kisofukushima, Kiso, Nagano 397, Japan

(Received 12 September 1996; Accepted 16 July 1997)

In 1985-1986 a study on terrestrial tardigrades was carried out on the northern slope of Mt. Fuji. As the third part of the taxonomic report on the Eutardigrada collected there, the present paper treats the families Macrobiotidae and Milnesiidae. Nine species of the two families were recorded, including a new species of *Pseudodiphascon.*

Key Words: Tardigrada, Eutardigrada, Macrobiotidae, Milnesiidae, *Pseudodiphascon fujiense* n. sp., Mt. Fuji, Japan.

Introduction

In 1985-1986, taxonomic and ecological studies on terrestrial tardigrades were carried out on the northern slope of Mt. Fuji. The author has already treated the families Eohypsibiidae and Calohypsibiidae in the first part of a taxonomic report on the class Eutardigrada (Ito 1991), and the family Hypsibiidae in its second part (Ito 1995). As the third and last part, the present paper reports the families Macrobiotidae and Milnesiidae. Eight species of Macrobiotidae and one species of the Milnesiidae were found. The former include a new species of *Pseudodiphascon.*

The tardigrades were extracted from surface soil, moss, and lichen using Baermann funnels. Their morphology was observed under a phase contrast microscope and a Nomarski differential interference microscope after the specimens had been mounted on slides in Gum Chloral.

Description

The sampling was carried out at 17 stations (Sts. 0-16) on the northern slope of Mt. Fuji. The location and a description of each sampling station are given in the second paper of this series (Ito 1995). All samples were taken by the author.

The type series of the new species, on slides, will be deposited in the National Science Museum (Nat. Hist.), Tokyo. Collecting data of all the species mentioned below are summarized in Table 1.

---

[1] *This work constitutes a part of a Ph.D. dissertation submitted to the Faculty of Agriculture, Tokyo University of Agriculture and Technology, and was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan* (*No. 63790256*).

Table 1. Number of tardigrades collected from each sampling station. Descriptions of each sampling station are given in a previous paper (Ito 1995). The abbreviations in the cells represent the kinds of habitats: SS, soil surface; MG, moss on the ground; LG, lichen on the ground; ML, moss on decaying log; MR, moss on rock; MT, moss on tree trunk; LT, lichen on tree trunk.

No. of stations	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Adorybiotus granulatus	MT: 11 LT: 10	MR: 1	–	–	MT: 16	ML: 31 MR: 16 MT: 5	–	ML: 2 MT: 69 LT: 4	–	MT: 62	–	MT: 11 LT: 1	–	–	–	–	–
Macrobiotus crenulatus	–	–	–	ML: 45	–	–	–	–	–	ML: 3	–	ML: 2 MT: 2	–	–	–	–	–
Macrobiotus hibernicus	–	ML: 11	–	–	–	–	–	ML: 4	–	–	–	–	–	–	–	–	–
Macrobiotus sp. cf. *hufelandi*	SS: 317 ML: 210 MT: 65 LT: 76	SS: 112 ML: 517 MR: 3256 MT: 382 LT: 158	SS: 135 MR: 12	SS: 19 ML: 81 MR: 3 MT: 11	SS: 45 ML: 1 MT: 21 LT: 1	SS: 207 ML: 39 MR: 13 MT: 39	SS: 593 MR: 1	SS: 19 ML: 243 MT: 147 LT: 9	SS: 375 ML: 3 MT: 63	SS: 125 ML: 12 MT: 19 LT: 1	SS: 38 MR: 6	SS: 96 ML: 17 MT: 30	SS: 45 LG: 80 LT: 11	SS: 734 ML: 2 MT: 44	SS: 275 MR: 131 MT: 314	SS: 381 MG: 390	LG: 89
Macrobiotus orcadensis	SS: 74 ML: 56 MT: 5 LT: 49	SS: 60 ML: 66 MR: 303 MT: 65 LT: 54	SS: 138 MR: 13	SS: 59 ML: 17 MT: 3	SS: 170	SS: 206 ML: 3 MT: 40	SS: 141	SS: 31 ML: 25 MT: 10 LT: 2	SS: 55 ML: 30 MT: 15 LT: 15	SS: 30 ML: 6	SS: 44	SS: 12 ML: 1 MT: 1 LT: 1	SS: 52 LG: 32	SS: 22 ML: 1	SS: 38 MR: 25 MT: 893	SS: 1 MG: 7	LG: 19
Macrobiotus richtersi	–	MR: 14 MT: 1	SS: 30	SS: 105 ML: 4	LT: 24	ML: 1 MR: 1 MT: 141	MR: 9	MT: 5 LT: 7	ML: 122 MT: 115 LT: 10	–	–	–	LG: 23	–	MR: 11 MT: 22	–	–
Minibiotus intermedius	SS: 134 ML: 53 MT: 16 LT: 147	ML: 2 MR: 421 MT: 137 LT: 20	MR: 2 MT: 4	SS: 4 ML: 158 MR: 78 MT: 40	MT: 25	SS: 2 ML: 24 MR: 43 MT: 13	MR: 7 MT: 6	SS: 2 ML: 26 MT: 37	MT: 3	SS: 1	–	SS: 2 MT: 6	SS: 53 LG: 85	SS: 1 ML: 1 MT: 1	MR: 2 MT: 62	–	LG: 439
Pseudodiphascon fujiense n. sp.	–	–	–	–	MT: 2	–	–	–	–	–	–	–	–	–	–	–	–
Milnesium tardigradum	ML: 1	SS: 1 ML: 14 MR: 31 MT: 1 LT: 1	–	–	ML: 4	–	SS: 1	SS: 1	–	–	–	SS: 1	SS: 1 LT: 1	SS: 4 ML: 2 MT: 1	MR: 4 MT: 18	SS: 10 MG: 14	LG: 16

–: Not collected.

Order **PARACHELA** Schuster, Nelson, Grigarick et Christenberry, 1980

Family **Macrobiotidae** Thulin, 1928
Genus ***Adorybiotus*** Maucci et Ramazzotti, 1981
Adorybiotus granulatus (Richters, 1903)
(Fig. 1)

Remarks. *Adorybiotus* is a monotypic genus. The present specimens are assigned to *A. granulatus* because they have spherical granules on the cuticle of the legs, large microplacoid and a sagittal crest of muscular insertions at the buccal tube. This crest is developed ventrally. The joint of this crest is not expanded. The distal end of the crest is not connected with the wall of the buccal tube. The present material has small constrictions on both the first and second macroplacoids. In Japan this species has been recorded previously from Mt. Choukai, Yamagata Prefecture (Utsugi 1991) and from Tokyo (Utsugi 1992a).

Genus ***Macrobiotus*** Schultze, 1834
Macrobiotus crenulatus Richters, 1904
(Fig. 2)

Remarks. Eggs were not collected, but the specimens are identified as *M. crenulatus* because of having: 1) large lunules with well developed dentation; 2) the body surface with large and distinctive pores. This species was recorded once, as *M. dentatus* Binda, 1974 from the high mountain area of Central Japan (Utsugi 1995).

Macrobiotus hibernicus Murray, 1911
(Fig. 3)

Remarks. This is the first record from Japan. This species is distinguishable by its short body length, the presence of three macroplacoids (first and second ones sometimes in contact) and the presence of fine punctation on the surface cuticle except for the dorsal median and ventral areas.

Until now, some workers have reported the presence of a microplacoid and others have reported its absence. The microplacoid could not be observed in the present material.

Macrobiotus sp. cf. ***hufelandi*** Schultze, 1834

Remarks. Many of the specimens have two macroplacoids and are easily assigned to the *M. hufelandi*-group (*sensu* Bertolani and Rebecchi 1993) by having a rather long first macroplacoid with a constriction and egg-cup-shaped egg ornamentations.

However, in the present study free eggs of this species were not collected. The egg surface was only observed through the adult body. The details of the egg protuberances were hardly visible. Therefore, the author cannot identify the present materials from among several sibling species of the *M. hufelandi*-group (Biserov 1990a, b; Bertolani and Rebecchi 1993).

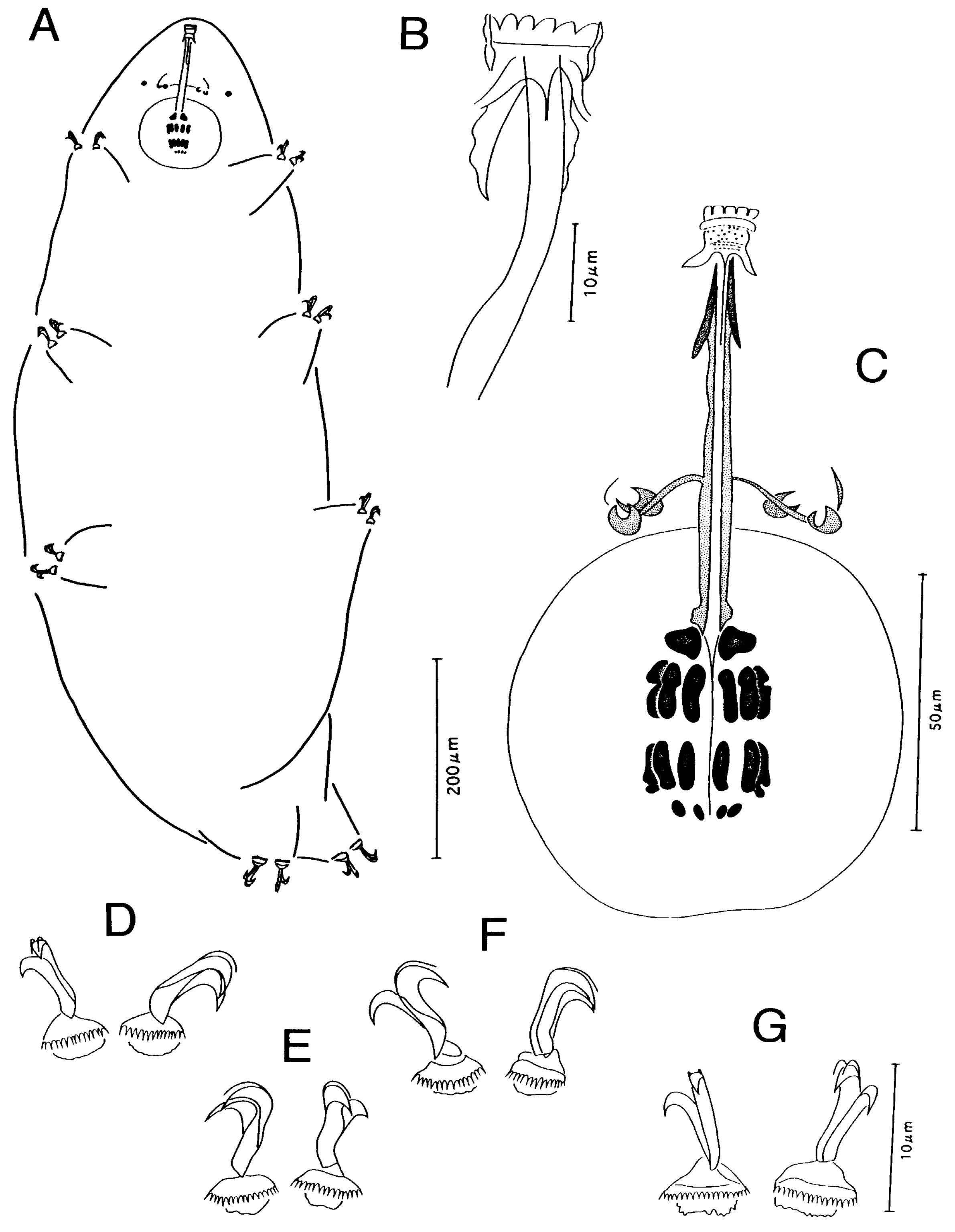

Fig. 1. *Adorybiotus granulatus* (Richters, 1903) - A, habitus, ventral view; B, anterior part of bucco-pharyngeal apparatus, lateral view; C, bucco-pharyngeal apparatus, ventral view; D, claws on leg I; E, claws on leg II; F, claws on leg III; G, claws on leg IV.

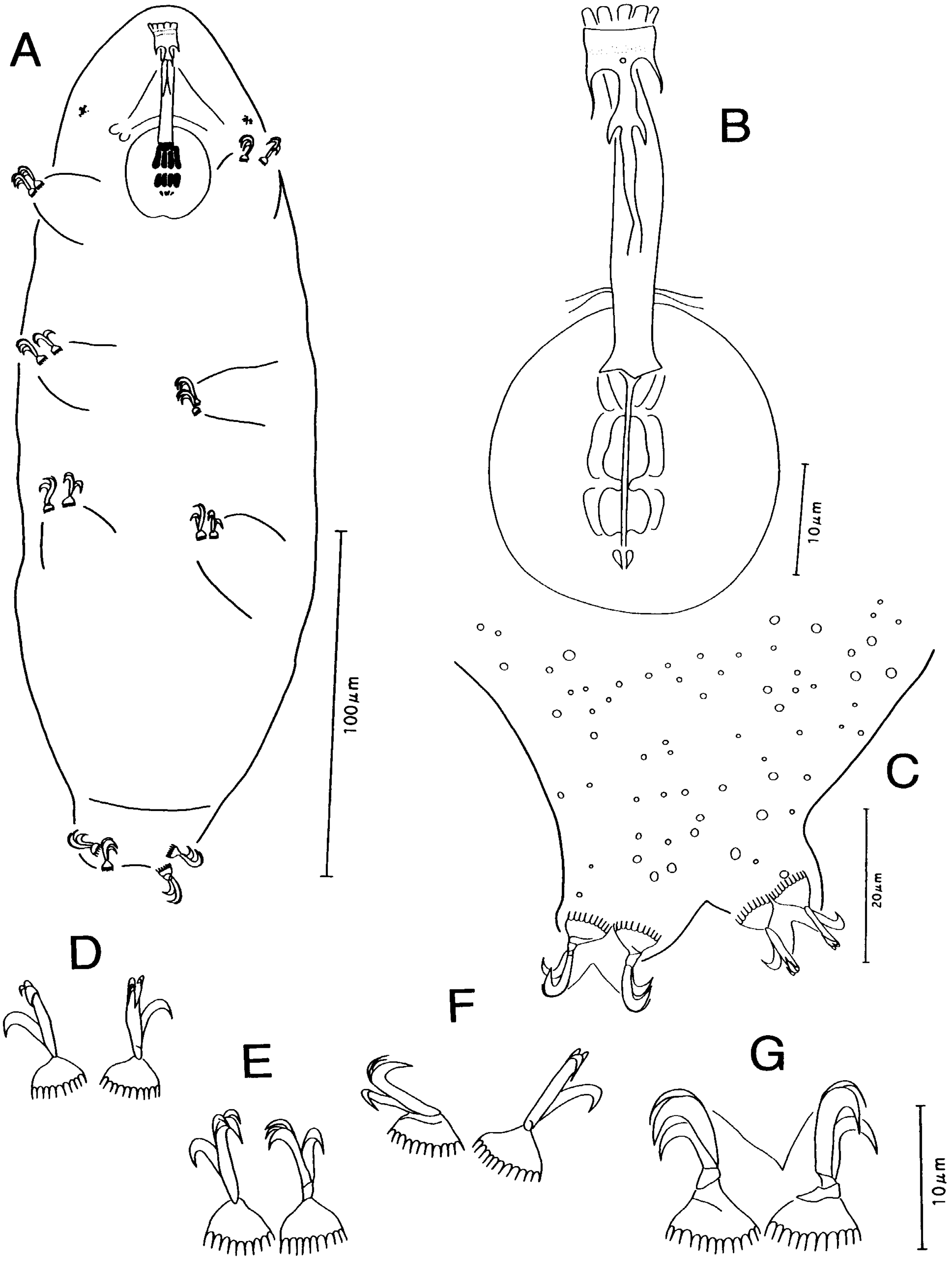

Fig. 2. *Macrobiotus crenulatus* Richters, 1904 - A, habitus, ventral view; B, bucco-pharyngeal apparatus, ventral view; C, legs IV and surface structure; D, claws on leg I; E, claws on leg II; F, claws on leg III; G, claws on leg IV.

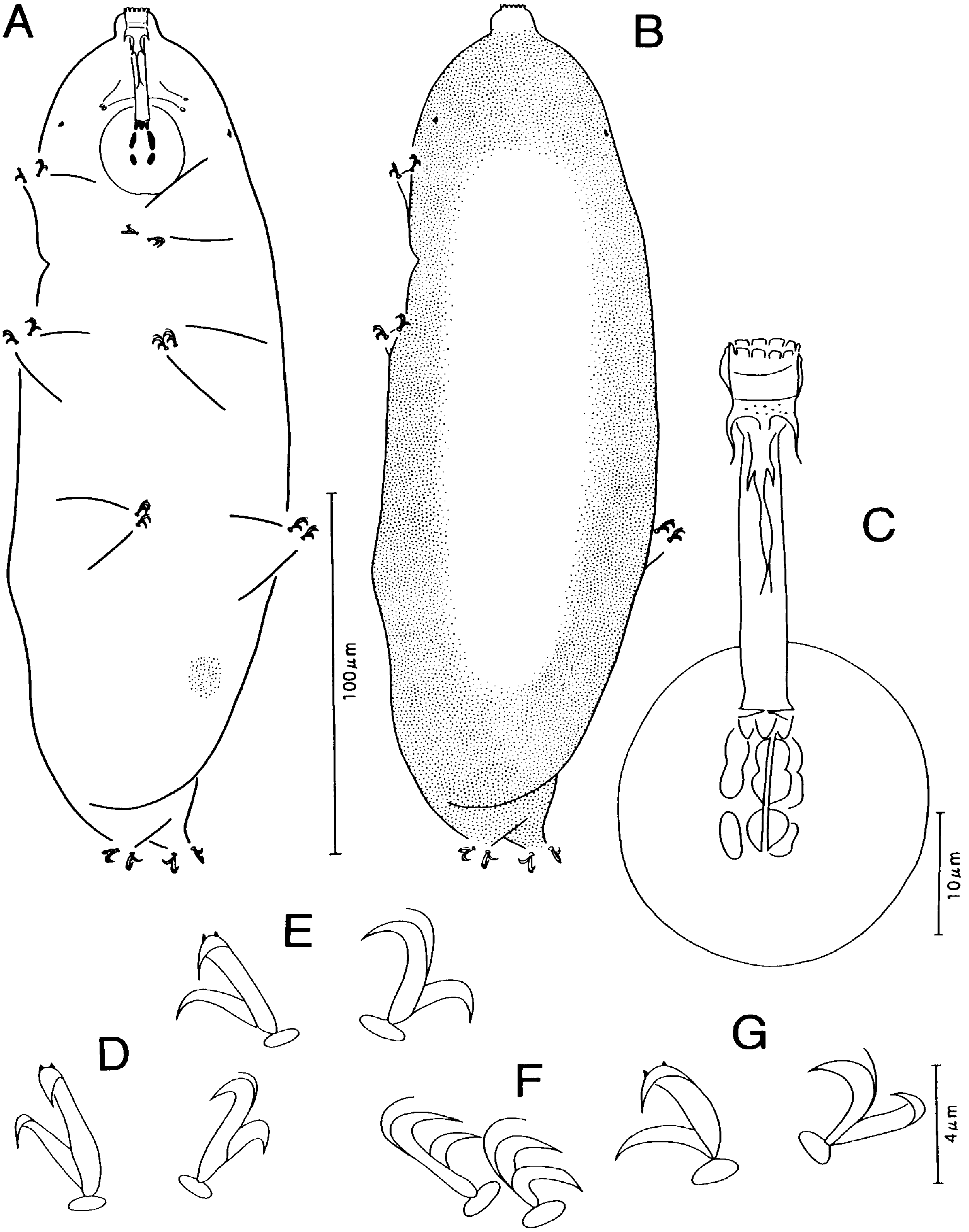

Fig. 3. *Macrobiotus hibernicus* Murray, 1911 - A, habitus, ventral view; B, distribution of fine punctation on dorsal cuticle; C, bucco-pharyngeal apparatus, ventral view; D, claws on leg Ⅰ; E, claws on leg Ⅱ; F, claws on leg Ⅲ; G, claws on leg Ⅳ.

Macrobiotus orcadensis Murray, 1907

Remarks. Specimens that belong to the *M. harmsworthi*-group (Ramazzotti and Maucci 1983), containing three macroplacoids and a microplacoid, were collected frequently in this study. They are identified as *M. orcadensis* in having: 1) conical egg protuberances with bifid apices; 2) the second macroplacoid being shortest.

In Japan, this species has been recorded previously from the Ryukyu Islands (Utsugi 1993).

Macrobiotus richtersi Murray, 1911

Remarks. This species is clearly distinguished by the following combination of characteristics: 1) the buccal tube is very wide and rigid; 2) of the three rod-like macroplacoids, the third is longer than the others; 3) the microplacoid is situated rather far from the last macroplacoid; 4) the egg surface is covered with conical protuberances and a round reticulation.

This species has already been recorded from many areas in Japan (Utsugi 1986, 1988, 1992b, etc.).

Genus ***Minibiotus*** Schuster, 1980

Minibiotus intermedius (Plate, 1889)

(Fig. 4)

Remarks. The present specimens are identified as *M. intermedius* because they have glanular macroplacoids, a very narrow buccal tube, and the egg ornamentation enclosed in a capsule. Pores on the cuticle are present in these specimens but they are very sparse and hardly visible.

Hatai (1956) reported this species from Japan; however, the author doubts his identification. Rod-like macroplacoids and a wide buccal tube are shown in his photograph (fig. 1). *Macrobiotus intermedius* (*sensu* Hatai) probably belongs to *M. harmsworthi*-group, judging from Hatai's (1956) drawing and photograph. In recent years Utsugi (1986, 1988, 1993, etc.) has reported this species from many points in Japan.

Genus ***Pseudodiphascon*** Ramazzotti, 1964

Pseudodiphascon fujiense sp. nov.

(Fig. 5, Table 2)

Measurements are given in Table 2.

Eyes: Absent. **Eggs**: Unknown. **Cuticle**: Smooth, without pores.

Bucco-pharyngeal apparatus: *Macrobiotus*-type (Pilato 1975). Ten peribuccal lamellae present. Both anterior and posterior bands of teeth of buccal armature present. Teeth of posterior band large and very distinct, standing in one row. Three obvious transverse crests present ventrally and dorsally. Buccal tube almost straight, with wide strengthening bar reinforced by spiral thickening posteriorly. Length of portion with spiral thickening 6.5 μm (18% of total length of buccal tube in the holotype). Pharyngeal bulb oval; ratio of length to width 1.24 (holotype); containing well developed apophyses, three rod-like macroplacoids, and a visible

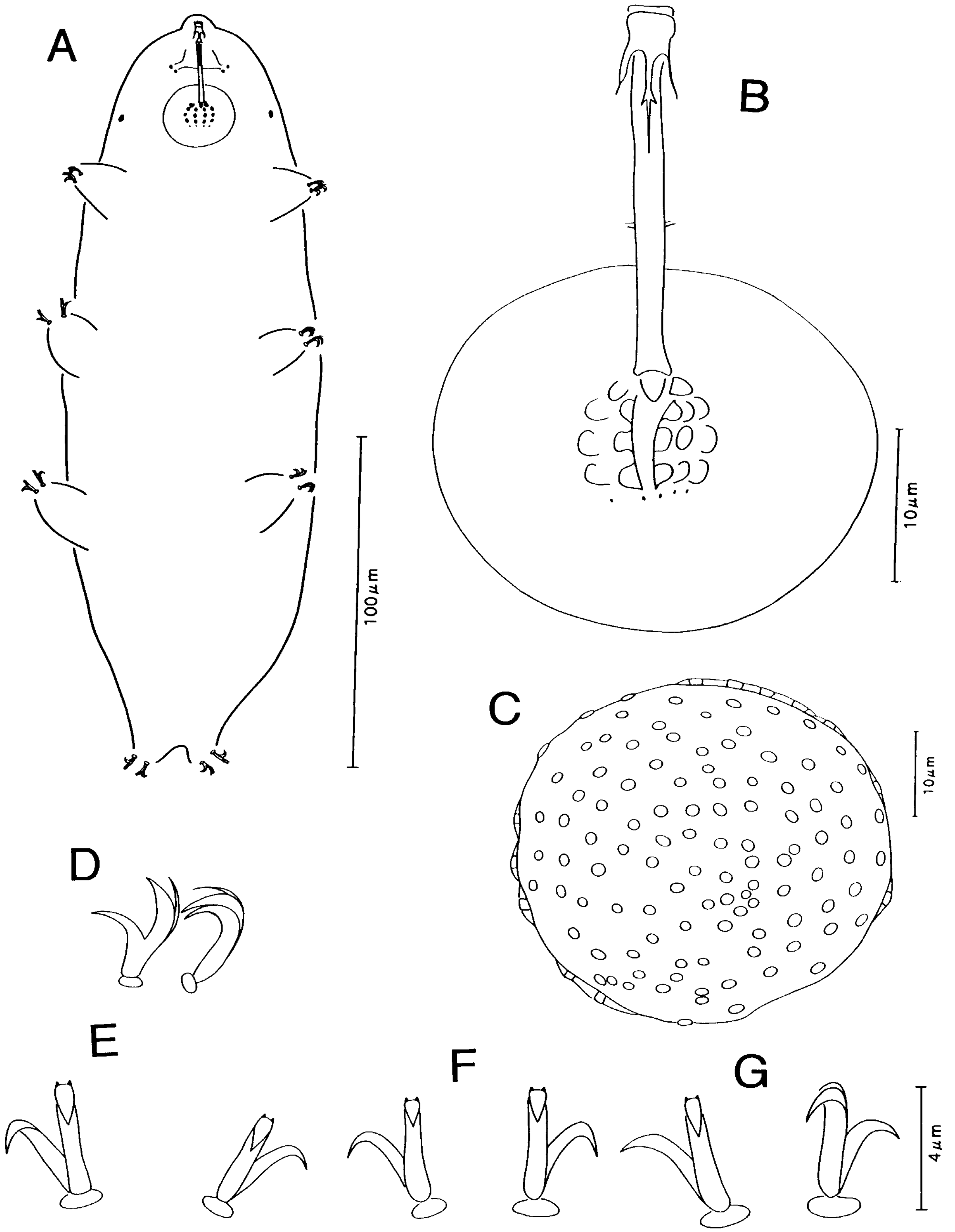

Fig. 4. *Minibiotus intermedius* (Plate, 1889) - A, habitus, ventral view; B, bucco-pharyngeal apparatus, ventral view; C, egg; D, claws on leg I; E, claws on leg II; F, claws on leg III; G, claws on leg IV.

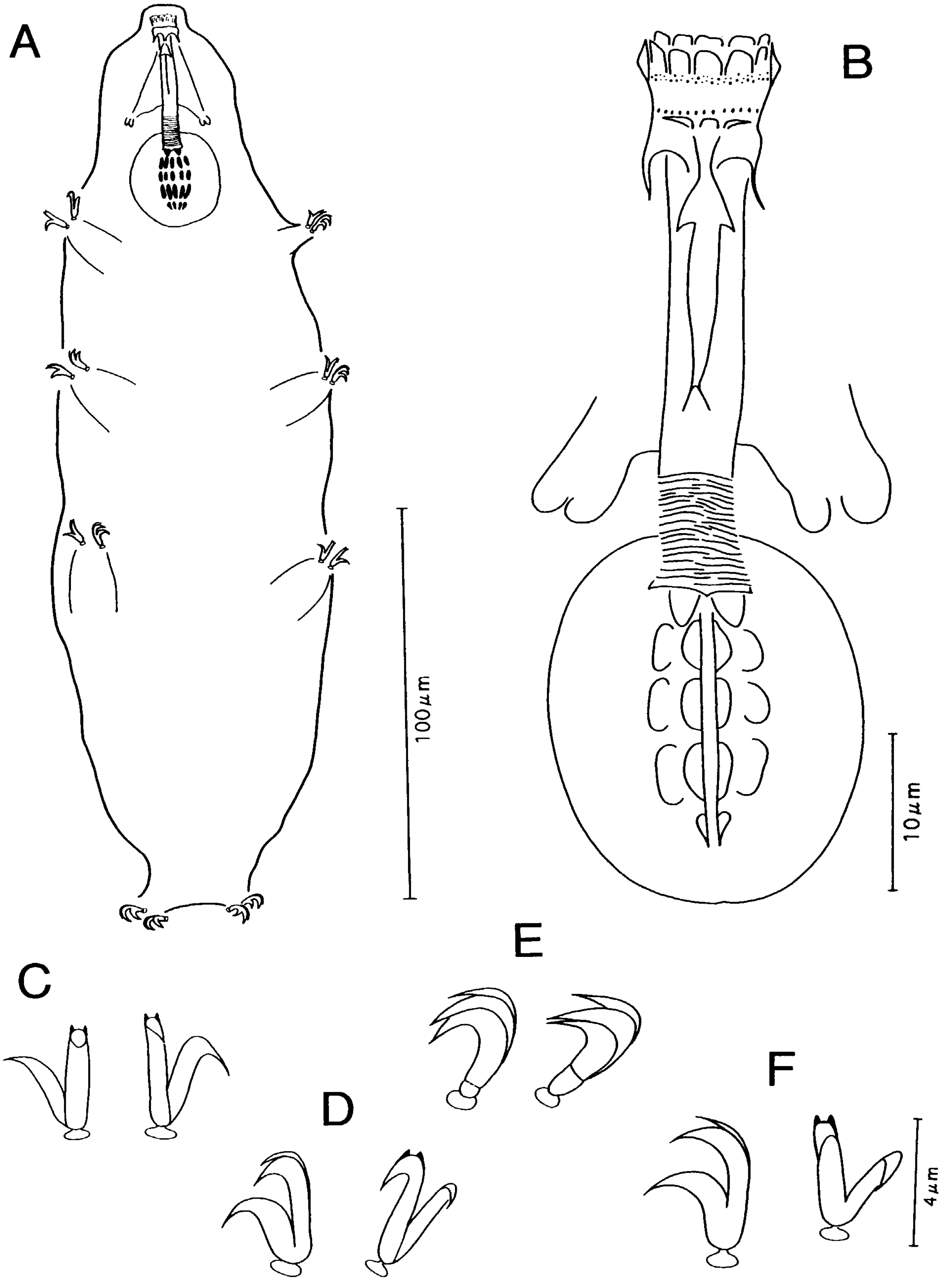

Fig. 5. *Pseudodiphascon fujiense* sp. nov. - A, habitus, ventral view; B, bucco-pharyngeal apparatus, ventral view; C, claws on leg I; D, claws on leg II; E, claws on leg III; F, claws on leg IV (A-B after holotype, C-F after paratype).

microplacoid. No septulum. Macroplacoids increasing in length from first to third, or almost equal. Microplacoid always shorter than first macroplacoid.

Claws: Of *hufelandi*-type (Ramazzotti and Maucci 1983), symmetrical; sequence 2-1-1-2. Shape and size of internal and external claws almost equal. Primary branches of internal and external claws of all legs with two accessory points. Lunule hardly visible at base of each claw, lacking dentation.

Material examined. Holotype (NSMT-Tg-42) and a paratype (NSMT-Tg-43) from moss on the trunk of beech tree *Fagus japonica* Maxim., St.4, in *F. japonica* forest with leaf litter accumulating thickly on ground and no moss or herbal vegetation, 13-IV-1986, Kamikuishiki-mura, Nishiyatsushiro-gun, Yamanashi Prefecture.

Remarks. This new species is related to *P. bindae* Christenberry et Higgins, 1979 (q.v.) and *P. arrowsmithi* Kathman et Nelson, 1989 (q.v.) in having an almost straight and rigid buccal tube and ten buccal lamellae. These common characters are very similar to those in *Macrobiotus*.

The new species can be distinguished from *P. bindae* in having: 1) lunules; 2) a wide buccal tube (ratio of width to length over 0.1, versus almost 0.05 in *P. bindae*); and 3) no eyes. It can be distinguished from *P. arrowsmithi* by the following combination of characteristics: 1) an oval pharyngeal bulb (almost round in *P. arrowsmithi*); 2) three rod-like macroplacoids slightly increasing in length from the first to the third or all almost equal (the second always shorter than the others in *P. arrowsmithi*); 3) the microplacoid distinctly shorter than the second macroplacoid (almost equal or larger than the second macroplacoid in *P. arrowsmithi*).

The remaining two species in the genus *Pseudodiphascon*, *P. inflexus* Arcidiacono, 1964 and *P. diphasconoides* (Iharos, 1969) (*P. inflexus*-group), are quite different from above three species (*P. bindae*-group) in having a very narrow and rather flexible buccal tube (Arcidiacono 1964; Iharos 1969). Neither author of these two species mentioned the existence of a stylet support or buccal lamellae. This type of buccal apparatus distinctly differs from that of *Macrobiotus* in having a rather flexible buccal tube.

The phylogenetic unity of *Pseudodiphascon* is doubtful because at least the two different types of buccal apparatus mentioned above are found in this genus.

Table 2. Measurements (μm) of the holotype and paratype specimens of *Pseudodiphascon fujiense* sp. nov.

	Holotype	Paratype
No. of Station	4	4
Body length	235.0	220.5
Body width (max.)	45.0	60.0
Width of mouth opening	6.6	4.9
Total length of buccal tube	35.3	36.4
Width of buccal tube	4.6	3.9
Length of pharyngeal bulb	26.3	22.1
Width of pharyngeal bulb	21.2	19.3
Length of first macroplacoids	4.2	3.0
Length of second macroplacoids	4.4	3.1
Length of third macroplacoids	4.7	4.7
Length of microplacoids	2.2	1.7

Confirmation of this suspition would require detailed reexaminations of *P. inflexus* and *P. diphasconoides* because their original descriptions are insufficient. Ramazzotti and Maucci (1983) pointed out that the condition of the type material of *P. inflexus* was not good for detailed observation.

Order **APOCHELA** Schuster, Nelson, Grigarick et Christenberry, 1980
Family **Milnesiidae** Ramazzotti, 1962
Genus ***Milnesium*** Doyère, 1840
Milnesium tardigradum Doyère, 1840

Remarks. *Milnesium* had been considered to be monotypic for a long time, but in recent years, three species have been added to the genus. The specimens from Mt. Fuji are in accord with the type species, *M. tardigradum*, in having the following characteristics: 1) the buccal tube is not very wide; 2) point a of the complex basal claw is smaller than point b; 3) six peribuccal lamellae are present; 4) the secondary branch of each claw is thin.

Mathews (1936/7) first reported this species from Japan. His drawing is consistent with an assignment to *Milnesium*, although his identification has been questioned (Rahm 1937). Later, Hatai (1956) recorded it from some areas in Japan along with drawings and microscopic photographs. These records are considered to be reliable. More recently, this species has been recorded many times from all around Japan (Hatai 1959; Watanabe *et al.* 1961; Utsugi 1986, 1988, etc.).

Acknowledgements

The author wishes to express his thanks to Prof. Dr. Jun-ichi Aoki of Yokohama National University, and Prof. Dr. Kazuo Utsugi and Mr. Hirokuni Noda of Tokyo Women's Medical College, for valuable advice and encouragement. Thanks are also due to Mr. Wataru Abe of the University of Tokyo for prividing valuable information about tardigrade literature and Prof. Dr. Masaki Kondoh of Shiraume Gakuen College for providing research space and supplies in the course of the extraction of the tardigrades.

References

Arcidiacono, R. 1964. Secondo contributo alla conoscenza dei Tardigradi dei Monti Nebrodi. Bollettino delle Sedute dell'Accademia Gioenia di Scienze Naturali in Catania 8: 187-203.

Bertolani, R. and Rebecchi, L. 1993. A revision of the *Macrobiotus hufelandi* group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. Zoologica Scripta 22(2): 127-152.

Biserov, V. I. 1990a. On the revision of the genus *Macrobiotus*. The subgenus *Microbiotus* s. str.: a new systematic status of the group *Hufelandi* (Tardigrada, Macrobiotidae). Communication 1. Zoologicheskiy Zhurnal 69(11): 5-17. [In Russian with English summary]

Biserov, V. I. 1990b. On the revision of the *Macrobiotus* genus. The subgenus *Macrobiotus* s. str. is a new systematic status of the *Hufelandi* group (Tardigrada, Macrobiotidae). Communication 2. Zoologicheskiy Zhurnal 69(12): 38-50. [In Russian with English summary]

Christenberry, D. and Higgins, R. P. 1979. A new species of *Pseudodiphascon* (Tardigrada) from Alabama. Transaction of the American Microscopic Society 98: 508-514.

Hatai, S. 1956. On the Japanese Tardigrada. Science Report of the Yokosuka City Museum 1: 1-12. [In Japanese with English summary]

Hatai, S. 1959. The distribution of *Milnesium tardigradum* Doy. in Japan. Science Report of the Yokosuka City Museum 4: 5-12. [In Japanese with English summary]

Iharos, G. 1969. Einige Angaben zur Tardigradenfauna Vietnams. Opuscula Zoologica Budapest 9(2): 273-277.

Ito, M. 1991. Taxonomic study on the Eutardigrada from the northern slope of Mt. Fuji, Central Japan, I. Families Calohypsibiidae and Eohypsibiidae. The Proceedings of the Japanese Society of Systematic Zoology 45: 30-43.

Ito, M. 1995. Taxonomic study on the Eutardigrada from the northern slope of Mt. Fuji, Central Japan, II. Family Hypsibiidae. The Proceedings of the Japanese Society of Systematic Zoology 53: 18-39.

Kathman, R. D. and Nelson, D. R. 1989. *Pseudodiphascon arrowsmithi*, a new species of tardigrade from British Columbia, Canada (Macrobiotidae: Eutardigrada: Tardigrada). Journal of the Entomological Society of British Columbia 86: 66-70.

Mathews, G. B. 1936/7. Tardigrada from Japan. Peking Natural History Bulletin, 2(4): 411-412.

Pilato, G. 1975. On the taxonomic criteria of the Eutardigrada. Memorie dell'Istituto Italiano di Idrobiologia Dott. Marco de Marchi, Pallanza 32 Suppl.: 277-303.

Rahm, G. 1937. A new ordo of tardigrades from the hot springs of Japan. Annotationes Zoologicae Japonenses 16: 345-352.

Ramazzotti, G. and Maucci, W. 1983. Il philum Tardigrada (III edizione riveduta e aggiornata). Memorie dell'Istituto Italiano di Idrobiologia Dott. Marco De Marchi, Pallanza 41: 1-1012.

Utsugi, K. 1986. Urban tardigrades in Kyushu. Zoological Science 3(6): 1110.

Utsugi, K. 1988. Tardigrades in Hokkaido area. Zoological Science 5(6): 1335.

Utsugi, K. 1991. Tardigrada. Pp. 16-20. *In*: Aoki, J. (Ed.) *Pictorial Keys to Soil Animals of Japan.* Tokai University Press, Tokyo, 201 pp. [In Japanese]

Utsugi, K. 1992a. Terrestrial tardigrades from mountain areas of Tokyo District. The Proceedings of the Japanese Society of Systematic Zoology 47: 69. [In Japanese]

Utsugi, K. 1992b. Urban tardigrades in the Tohoku area. Zoological Science 9(6): 1297.

Utsugi, K. 1993. Terrestrial tardigrades in the Ryukyu area. Zoological Science 10(Supplement): 174.

Utsugi, K. 1995. Terrestrial tardigrades from mountain areas of Japan. Zoological Science 12(Supplement): 36.

Watanabe, Y., Sasaki K. and Taira, K. 1961. Preliminary survey of tardigrades on dead bamboo-leaves from Chiba Prefecture. Science Report of the Yokosuka City Museum 6: 93-96.